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(54) **Title:** CHITIN MICROPARTICLES AND THEIR MEDICAL USES

(57) **Abstract:** Chitin microparticle compositions and their uses in the treatment of conditions such as allergy, infection and cancer are disclosed.

Chitin Microparticles and Their Medical Uses

Field of the Invention

The present invention relates to chitin microparticles and their medical uses, in particular in the treatment of allergy, conditions that would benefit from an up-regulation of the cell mediated immune system and conditions that would benefit from an up-regulation of natural killer (NK) cell activity and/or the secretion of interferon-γ (IFN-γ).

Background of the Invention

The alveolar macrophage is the most abundant leukocyte in the lumen of the alveolus and is central to the innate immune system of the lung by promoting phagocytic clearance and by the secretion of cytokines that promote an effective cell mediated immune response to inhaled particulates including microbes and pathogens. The principle cytokines produced during phagocytosis are IL-12, TNF-α, and IL-18. These macrophage cytokines subsequently induce IFN-γ production by NK cells and Th1 lymphocytes. IFN-γ acts synergistically with these cytokines to promote a Th1 cell mediated immune response and also down-regulate the production of Th2 cytokines, in particular IL-4 and IL-5 which are strong mediators of allergy.

Studies by Shibata *et al* (1-4), have shown that oral delivery of 1-10μm phagocytosable chitin particles results in an elevation of Th1 cytokines in mouse spleen cell cultures. The effect was specific to the particulates as no elevation was produced by soluble chitin. It could also be reproduced in 1μm polystyrene

microspheres coated with *N*-Acetyl-D-Glucosamine, which is the main component of chitin. It was also demonstrated that oral administration of chitin down-regulates serum IgE and lung eosinophilia in a murine model of ragweed allergy (1).

Shibata *et al* have also developed a mouse model of allergic airway inflammation and orally administered chitin preparations to the mice (Shibata 2000). Ragweed-specific IgE levels were significantly reduced after daily oral administration of chitin to ragweed-sensitised mice, before and during immunisation. Bronchioalveolar lavage (BAL) cells were harvested 14 days after immunisation and a reduction in the levels of eosinophil and lymphocyte levels was observed after chitin treatment. Lung inflammation was determined histologically 14 days after immunisation and the peribronchial, perivascular and total lung inflammation were inhibited in the chitin-treated group.

When chitin was administered prophylactically to mice who were subsequently administered ragweed, IL-4, IL-5 and IL-10 production was significantly reduced and low but significant levels of IFN-γ were detected.

Chitin also has a prophylactic effect when administered to C57BL6 mice, which are higher responders for cell-mediated immunity/Th1 responses, but lower responders for allergic responses compared with BALB/c mice.

When ragweed-sensitised mice were treated simultaneously with ragweed and chitin, the levels of IL-4, IL-5 and IL-10 produced were significantly reduced compared to those stimulated by ragweed alone.

However, while Shibata *et al* disclose the use of chitin microparticles for the treatment of allergy, the compositions are administered orally as a supplement to activate macrophages and prophylactically strengthen the immune system in the absence of recurrent bacterial infections that are decreasingly common in industrialised countries.

Japanese Patent Application No:19997-0087986 A (Unitika Limited) discloses the use of deacetylated chitin particles in the form of powders, granules or fibres for delivery to the nasal mucosa. The chitin particles have an effective particle diameter of 20 to 250 microns and are proposed for the treatment of allergic symptoms at an inflammatory site such as pollinosis.

US Patent No: 5,591,441 9 (Medical Sciences Research Institute) concerns the use of particulate compositions for providing protection against microorganism infection and biological warfare agents. The compositions are delivered intravenously with the aim of providing a short lived increased in *in vivo* peroxide levels to kill the microorganisms.

More generally, existing treatments for allergies typically involve the use of steroids to depress the immune system. There are undesirable side effects with steroid therapy. Synthetic drugs, such as steroids are expensive to manufacture, involving a complex process which requires complex quality control and GMP standards to meet requirements of Health and Safety Authorities. In view of these factors, it remains a problem in the art in finding effective treatments for allergy.

Pseudomonas aeruginosa, an opportunistic pathogen, is a leading cause of life threatening infections in immunocompromised individuals and is a major risk to patients on ventilator support and many disease conditions in which there is a reduction in lung function and a reduced ability to clear infections. Each year, over two million patients die as a result. A report on the incidence of hospital-associated infections places *P. aeruginosa* among the three most frequently reported pathogens (5). *P. aeruginosa* is also a common cause of chronic and life threatening pulmonary infection in cystic fibrosis patients. Recent reports list *P. aeruginosa* among the most serious antibiotic-resistant bacteria and one for which effective vaccines are needed (6).

Streptococcus pneumoniae is a ubiquitous pathogen and responsible for a high proportion of cases of pneumonia (both lobar and bronchopneumonia) and one of the leading causes of illness and death among young children, the elderly and those with an impaired immune system as the result of diseases, such as AIDS, or immunosuppressive therapy, such as for bone marrow transplantation. The invasive form of Streptococcal infection, in which the bacteria disseminate into the blood and other organs leads to very serious complications. Each year in the United States, pneumococcal disease is estimated to cause 3000 cases of meningitis, 50,000 cases of bacteraemia, 125,000 hospitalisations and 6,000,000 cases of Otitis media.

There is a growing concern about the emergence of antibiotic resistant strains of *S. pneumoniae* and there

is a considerable amount of research into new treatments and vaccines.

Summary of the Invention

Broadly, the present invention relates to the use of chitin microparticle (CMP) preparations for treating disorders by delivering the microparticles intranasally to the sinuses and upper respiratory tract, e.g. using an intranasal spray, or by inhalation, e.g. targeting alveolar macrophages in the lungs. The chitin microparticle compositions are different to many of those disclosed in the prior art, typically having particle sizes of less than 10μm.

The macrophage has a central control function in the innate immune system of the lung by promoting phagocytic clearance of particles and by processing the presentation of inhaled allergens to lymphocytes and by secretion of cytokines that promote an effective cell mediated immune response to inhaled particulates including microbes and allergenic substance. In particular, the present invention discloses that intranasal delivery of chitin microparticles is particularly effective in reducing a number of parameters indicative of inflammation, thus providing an alternative to steroid treatments.

The work disclosed herein arises from the finding that the intranasal delivery of chitin microparticles to mouse models of allergy produced by *Aspergillus fumigatus* (Afu) and *Dermatophagoides pteronyssinus* (Der p) is particularly effective in reducing levels of peripheral blood eosinophilia, serum total IgE, Afu-specific IgG1, the cytokine IL-4, GM-CSF, and airway hyperresponsiveness (AHR), as well as increasing levels of the cytokines IL-

12, IFN-γ and TNF-α. Intranasal pre-treatment of chitin microparticles was effective in preventing the progression of infection by *P. aeruginosa* or *S. pneumoniae* in mice infected with these pathogens. In addition, CMP have been shown to enhance the TNFα and IFNγ response of activated human leukocytes.

Accordingly, in a first aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for treating allergy, wherein the medicament is delivered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from allergy, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Examples of allergies that can be treated according to the present invention include seasonal respiratory allergies, commonly referred to as hay fever; allergy to aeroallergens including house mite dust, fungal spores, grass pollens, tree pollens and animal danders; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema and food allergies; dermatitis such as atopic dermatitis.

In a further aspect, the present invention provides a composition comprising a chitin microparticles composition and an allergen. These compositions can be employed in the treatment of allergies and allergic

symptoms, such as anaphylactic shock, which are associated with conventional desensitisation therapy. Oral application of IL-12 has been shown to suppress anaphylactic reactions and so administering an allergen with a CMP composition should help to moderate the anaphylactic reactions arising during desensitisation therapy designed to build up tolerance to an allergen. Allergens can be readily extracted from food and are commercially available as they are used in the diagnosis and treatment of allergy. One particular application of this aspect of the invention is in the treatment of food allergy. Examples of common food allergens include milk, wheat, gluten, eggs, nuts or shellfish, and the skilled person will be able to formulate these with the CMP composition for delivery to a patient.

In an alternative aspect, the present invention provides a kit comprising:

(a) a chitin microparticle composition; and
(b) an allergen;

for simultaneous or sequential administration to a patient.

One further specific embodiment involving the treatment of allergy is in the treatment of horses, and particularly thoroughbred horses, which have a tendency to suffer from allergic conditions such as asthma or recurrent lung infections.

In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of conditions that would benefit from the up-regulation of the cell-mediated immune system, wherein the medicament

is administered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition that would benefit from the up-regulation of the cell-mediated immune system, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

Thus, in this aspect of the invention, the CMP composition can be used to strengthen the immune system of an individual. Conditions that benefit from the up-regulation of the cell-mediated immune system include the treatment of microbial infections, including bacterial infections, fungal infections and viral infections, particularly among vulnerable patient groups such as the elderly, premature babies, infants, transplantation patients, immunosuppressed patients such as chemotherapy patients, hospital patients at risk of opportunistic infection, patients on ventilators, cystic fibrosis patients and patients with AIDS. The invention is particularly applicable to the treatment of ear, nose, throat and lung infections.

Specific examples of bacterial infection include the treatment of infection by microorganisms such as *Pseudomonas aeruginosa, Streptococcus* species such as *Streptococcus pneumoniae, Streptococcus pyrogenes, Streptococcus agalactiae, Haemophilus influenza, Klebsiella pneumoniae, Yersinia enteocolitica, Salmonella, Listeria, Mycobacterial infections including Mycobacterium tuberculosis, Mycobacterium leprae,*

parasitic infections including *Leishmania* species and *Schistosoma* species.

One condition caused by microbial infection, typically by *Streptococcus pneumoniae*, is recurrent ear infections such as Otitis media. These conditions occur in children and adults and are currently treated using antibiotics. It would be advantageous to use the chitin microparticle compositions of the invention to treat these conditions and reduce the need for antibiotics.

The preparations of the invention can be used in the treatment of tuberculosis either to treat an existing infection or to protect vulnerable patient groups from infection.

Other examples of microbial infections include bacterial pneumonias, such as ventilator-associated pneumonia, and cystic fibrosis associated infections.

Examples of fungal infections include fungal infections such as invasive pulmonary aspergillosis and invasive pulmonary candidiasis, *Pneumocystis carinii* pneumonia, *Coccidioides* and *Crytococcus* infections, e.g. in immunosuppressed patients.

Examples of viral conditions treatable according to the present invention include pulmonary viral infections such as respiratory syncytial virus bronchiolitis, especially in infants and the elderly, or influenza virus, or rhino virus. Numerous studies have shown that during the progression of AIDS, mononuclear cells lose their ability to secrete IL-2, IL-12 and IFN-γ and produce increased levels of IL-4, which allows the HIV virus to

proliferate. Therefore treatment with CMP, given intranasally or by inhalation, will be useful in reducing the progression of HIV infection by restoring IL-12 and IFN-γ levels.

In a further aspect, the present invention provides the use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of conditions treatable by up-regulation of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, wherein the medicament is administered intranasally or by inhalation.

In an alternative aspect, the present invention provides a method of treating a patient suffering from a condition treatable by up-regulation of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, the method comprising administering to the patient a therapeutically effective amount of a chitin microparticle preparation, wherein the CMP preparation is administered intranasally or by inhalation.

An example of a condition treatable in this aspect of the invention is cancer, and especially lung cancer, lung carcinoma or nasal-pharyngeal carcinoma.

Preferably, the medicaments set out above are for administration to humans. Preferred patient groups for intranasal treatment with CMP would include those suffering from seasonal allergic rhinitis and sinusitis, or chronic respiratory allergies such as house dust mite allergy and who are currently taking steroids or antihistamines. Other groups include hospitalised

patients being treated for chronic lung disorders including infections and lung carcinomas.

Chitin is a polymer of N-acetyl-D-glucosamine and has a similar structure to cellulose. It is an abundant polysaccharide in nature, comprising the horny substance in the exoskeletons of crab, shrimp, lobster, cuttlefish, and insects as well as fungi. Any of these or other sources of chitin are suitable for the preparation of CMP preparations for use according to the present invention.

Preferably, chitin is produced by physically reducing it, e.g. by sonication or milling, to particles having a diameter of less than 50μm, more preferably less than 40μm, still more preferably less than 20μm, more preferably less than 10μm and most preferably less than 5μm. As we have found that the effects caused by chitin microparticles are size dependent, it is preferred that the chitin microparticles have average diameters which are less than 10μm. An upper limit of chitin particles size may be functionally defined by macrophages not recognising the particles. The lower size limit is less important, but preferably the particles are at least 1μm in diameter. The lower size limit is functionally defined by the chitin particles becoming soluble and hence also not being recognised by macrophages. Particles size and size distribution can readily be determined by the skilled person for example using flow cytometry or a microscope. Alternatively or additionally, the chitin microparticles can be made by coating carrier particles, e.g. formed from a biocompatible material such as polystyrene or latex, with *N*-Acetyl-D-Glucosamine, chitin or a fragment thereof, to

form particles having the sizes as defined above, and these compositions are included within the term chitin microparticle composition as used herein.

It should be recognised that in a composition, the chitin microparticles will have a distribution of sizes, typically a normal distribution, and that not all particles within a population will necessarily meet these size limits. However, within a population of chitin microparticles forming a CMP preparation, preferably at least 60%, more preferably at least 75%, more preferably at least 90%, and more preferably 95% and most preferably at least 99%, of the chitin particles have a size distribution within the limits set out above.

In a further aspect, the present invention provides a delivery device comprising a reservoir of chitin microparticles as defined herein, and a delivery orifice adapted to locate in a patient's mouth or nose, wherein the patient can place the delivery orifice in the mouth or nose to administer the chitin microparticles. In some embodiments the device may comprise a valve between the reservoir and the delivery orifice, such that the valve can be operated to control delivery of the chitin microparticles. The microparticles may be drawn into the nose to the sinuses and upper respiratory tract or through the mouth to the alveolar macrophages by inhalation and/or by a propellant. A particularly preferred form of device is a nasal spray bottle containing a CMP preparation and optionally a carrier, the spray bottle having a neck adapted for nasal delivery.

In addition to chitin microparticles, the CMP preparations can comprise one or more of a pharmaceutically acceptable excipient, carrier, propellant, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1% to 1.0% (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual, e.g. providing alleviation of allergy or another condition or prophylaxis for an acceptable period. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to

be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980 and Remington's Pharmaceutical Sciences, 19th edition, Mack Publishing Company, 1995. The compositions are preferably administered in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight. By way of example, this could be achieved using a nasal delivery bottle to deliver 4-8 doses of approximately 0.25ml of a 5 mg/ml solution of CMP particles.

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures.

Brief Description of the Figures and Tables

Figure 1 shows the results of treatment of Afu challenged mice, with 17μg CMP which produced a significant decrease ($p<0.05$) in peripheral blood eosinophilia.

Figure 2 shows the results of treatment of Der p and Afu challenged mice with 4 daily doses of 25μg CMP which produced a significant decrease ($p<0.05$) in peripheral blood eosinophilia.

Figure 3 shows a reduction in serum total IgE ($p<0.0005$) of Afu challenged mice, after treatment with 17μg/day of CMP.

Figure 4 shows a reduction in serum total IgE ($p<0.0005$) of Afu challenged mice, after treatment with 5 daily doses of 17μg CMP and after re-challenge with allergen 1 week later.

Figure 5 shows the results of treatment of Der-p challenged mice, with 5 daily doses of 25μg CMP which produced a significant decrease ($p<0.005$) in total serum IgE.

Figure 6 shows a reduction in Afu specific IgG1 ($p<0.01$).

Figure 7 shows a reduction in Afu specific IgG1 ($p<0.001$) after treatment with 5 daily doses of 17μg CMP, and after re-challenge with allergen 1 week later.

Figure 8 shows a reduction in airway hyperresponsiveness ($p<0.01$) in mice re-challenged with the Afu antigen after treatment with CMP.

Figure 9 shows a reduction in AHR ($p<0.01$) in mice challenged with Afu antigen, after 4 days of CMP treatment.

Figure 10 shows a reduction in AHR, in mice challenged with the Der p antigen after treatment with CMP, to increasing concentrations of methacholine.

Figure 11 shows a reduction in AHR 3 days after treatment with 25μg CMP preceded by allergen challenge (Der-CMP(0), $p<0.001$) and re-challenge 4 days later (Der-CMP(4), $p<0.001$).

Figure 12 shows a reduction in AHR in mice challenged with Der p, after 4 days of treatment with varying doses of CMP, in response to methacholine exposure.

Figure 13 shows lung sections differing in the degree of inflammation and obstruction after CMP treatment of Afu sensitised mice.

Figure 14 shows the survival of mice pretreated with CMP or control (PBS), in response to *P. aeruginosa* infection.

Figure 15 shows the time-course for the clearance of *S. pneumoniae* from infected murine lung.

Figure 16 shows the time-course for the appearance of *S. pneumoniae* in the blood of infected mice.

Figure 17 shows the enhancement by CMP on TNFα production by LPS activated CD14 monocytes.

Figure 18 shows the enhancement by CMP on TNFα production by LPS activated CD14/CD16 proinflammatory monocytes.

Figure 19 shows enhanced production of IFNγ from PMA/Ionomycin stimulated human T-lymphocytes by CMP.

Table 1a indicates increases in the cytokines IL-12, IFN-γ and TNFα in spleen cells of mice challenged with Der p and Afu allergens, in response to treatment with CMP.

Table 1b indicates an increase in the cytokine GM-CSF in spleen cells of mice challenged with Der p, in response to treatment with CMP.

Table 1c indicates a decrease in the geometric mean fluorescence of the cytokine IL-4 in response to treatment with CMP.

Detailed Description

Materials and Methods

Chitin microparticles delivered intranasally represent a new approach to stimulating cell mediated immunity and promoting anti-inflammatory responses in inflamed tissues. The present invention has the considerable advantage that macrophages of the upper respiratory tract or alveolar macrophages can be directly targeted with chitin microparticles of the correct size using an intranasal spray and inhalation delivery respectively.

Two models of allergy, a model of pathogenic lung infection and a model of bacterial infection have been established in the mouse. In addition, an *in vitro* assay utilizing human monocytes or lymphocytes has been developed, in order to demonstrate the efficiency of the present invention.

The parameters measured in the allergy models are serum IgE and IgG1, peripheral blood eosinophilia, and AHR, which are all significantly elevated in the mouse models of allergy to Afu and Der p allergens and are all significantly reduced by intranasal treatment with CMP. Levels of the cytokines IL-12, IFN-γ and TNF-α which are reduced in the mouse model of allergy to Der p are all increased by intranasal treatment with CMP and levels of GM-CSF and IL-4 are reduced. The proposed mode of action is that the CMP are bound by the mannose receptors of macrophages in the nasal mucosa and alveolae, which

stimulates macrophages and dendritic cells to generate IL-12, TNF-α and IL-18, and reverses the suppression of IL-12 produced by allergen challenge, returning the levels to those observed in non-allergic mice. This leads to the generation of IFN-γ by NK cells and Th1 lymphocytes. The reduction in GM-CSF and IL-4 is indicative of a modulation of the immune response from Th2 to Th1. In fact, all these cytokines and particularly IFN-γ, promote a shift in the populations of T lymphocytes from Th2 to Th1. GM-CSF is a Th2 cytokine that promotes the differentiation, activation and survival of eosinophils. This culminates in the observed reduction in serum IgE and eosinophilia, which are major components in allergy.

In the mouse model of pathogenic lung infection the survival of mice pre-treated with CMP was measured over 10 days in response to infection with *P. aeruginosa*. Mice pre-treated with CMP showed a significantly improved survival rate over mice which received no pre-treatment with CMP.

In the mouse model of bacterial infection the time-course for the clearance of *S. pneumoniae* from the lungs of infected mice was measured over 24h. Mice pre-treated with CMP showed significantly ($p<0.001$) lower bacterial colony forming units (cfu) in the lungs compared to PBS pre-treated mice. The time-course for the appearance of *S. pneumoniae* in the blood was measured over 48h and blood bacteraemia in CMP pre-treated mice was significantly less at 24h ($p<0.005$) and 48h than that of PBS pre-treated mice.

The production of TNFα in response to LPS stimulation was measured in human monocytes and IFNγ levels were measured in human T-lymphocytes in response to PMA/Ionomycin stimulation. In both assays, the addition of CMP enhanced cytokine production. This effect, observed in human cells, is consistent with the concept that CMP prime monocytes and phagocytes through binding to the mannose receptor or other carbohydrate receptors, followed by phagocytosis of the CMP. Activation by a microbial product such as lipo-polysaccharide (LPS), enhances the responsiveness of these monocytes, an effect mediated by phagocyte-derived cytokines such as IL-12. CMP can also promote the activity of Th1 lymphocytes, consistent with the concept that CMP promotes a Th1 cell mediated immune response.

Chitin Microparticle Suspension Preparation (CMP)

Chitin microparticles were prepared from purified chitin (Sigma-Aldrich, Poole, UK) by sonication of a suspension of 10mg/ml in endotoxin free PBS at maximum output for 20min with cooling on ice every 5min. The slurry was centrifuged at 1000xg for 10min to remove large particles and the microparticles were collected by centrifugation at 4000xg and washed 3 times with PBS to remove any solubilized chitin. The supernatant contained a uniform suspension of small particles as judged by light microscopy using a haemocytometer with 50μm squares and were comparable in size to 1μm latex spheres (Polysciences, Inc., Warrington, Pennsylvania, USA). Particles less than 5μm in diameter were quantified with a Celltac Hematology Analyser (Nihon Kohden, Inc.). Preparations were found to contain 99.9% microparticles less than 5μm in diameter and at a concentration in the

order of 10^{11}/ml. Endotoxin was measured by Limulus Amebocyte Lysate Assay (BioWhittaker Co,) and shown to be <1 EU/ml.

Mouse Models of Allergy

Allergen extracts

Aspergillus fumigatus (Afu) was grown in a synthetic medium (M199, Sigma Chemicals) as a stationary culture for 1 week at 37°C. Arrunda *et al*, demonstrated that the expression of Asp f1, a major allergen, is maximal after 1 week and tends to diminish during longer incubation periods (7). The 1 week culture was killed by adding 0.1% Thimerosal for 12 hours. The culture was filtered through glass wool and finally through a 0.45μm membrane to remove all particulates and possible spores and then dialysed with 3 buffer changes against water. The dialysate was lyophilised to give a brown powder.

A major band at 18kDa corresponds to Asp f 1. A band corresponding to Asp f 2 (37kDa) is also evident. The 18kDa band was N-terminal sequenced giving the sequence ATWTCINQQLNP, corresponding to the N-terminal sequence for Asp f 1.

It was also demonstrated by ELISA that the 1-week culture filtrate (1wcf) was recognised by human serum from Afu-allergic patients obtained from the National Institute of Biological Standards and Control.

Standardized *Dermatophagoides pteronyssinus* (Der p) extract (Greer Labs, Lenoir, North Carolina, USA) containing 10000 Allergy Units (AU)/ml was diluted into sterile endotoxin free PBS.

Sensitisation

Female C57BL/6 mice were sensitised by 4 weekly i.p. injections of a mixture of allergen extract (68 AU Der p; 200μg Afu) with alum in 100μl of sterile PBS.

Allergen challenge and treatment with CMP

Sensitised mice were anaesthetized with isoflurane and challenged with 50 allergy units of Der p extract, or 10μg Afu allergen extract, in PBS given intranasally followed by intranasal doses of PBS or CMP or a particulate control (PC) of 1μm polystyrene beads in 50μl given 1-2 hours later. In a separate experiment it was shown that approximately 50% of FITC-labelled micro-beads given intranasally could be recovered from the lungs after 30min.

Peripheral blood eosinophils

Blood was collected from the tail vein of the mice (n=4-8 /group) for estimation of eosinophils. The total leukocyte count was determined by an automatic cell counter and the proportion of eosinophils was determined by differential counting of May-Grunwald-Giemsa stained blood smears. Results are expressed as 10^6 cells/ml.

Serum IgE and Afu-specific IgG1

Total serum IgE was measured by sandwich ELISA (BD PharMingen, Cowley, UK) in blood serially diluted from a maximum dilution of 1:20 to give values, which were linear with respect to a standard curve of mouse IgE. Results are expressed in μg/ml. Antigen-specific IgG1 was measured by ELISA using 96-well plates coated with allergen extract. Antibody was detected with HRP-labelled anti-mouse IgG1. Results are expressed as relative

absorbance units (OD450).

<u>Intracellular cytokine staining.</u>

After treatment, mice were humanely sacrificed by CO_2 asphyxiation and their spleens removed and homogenized in PBS. The homogenate was filtered and red blood cells lysed with ammonium chloride lysing reagent (BD Pharmingen, Cowley, UK) and fixed with 4% (v/v) paraformaldehyde for 20min. The cells were washed with PBS supplemented with 3% heat inactivated fetal calf serum with 0.1%(w/v) sodium azide (FSB), re-suspended in 10% DMSO(v/v) in FSB and stored at -80°C.

Cells were permeabilized with Cytoperm wash buffer (CPB, BD Biosciences, Cowley, UK) for 15min at 4°C and aliquots of 10^6 cells were blocked by incubation for 30min at 4°C with CPB supplemented with 1%(v/v) rat IgG. Intracellular cytokines were stained with 1μg PE-conjugated anti-mouse cytokine monoclonal antibody (BD Biosciences, Cowley, UK) incubated for 60min at 4°C. The cells were washed with CPB followed by FSB and re-suspended in 500μl FSB.

Flow cytometry was performed with a FACScan flow cytometer (Beckton Dickinson, Mountain View, California, USA) using CellQuest software. Data were collected for 20000 cells. The average FSC of spleen cells was 100 in all cases. Stained cells (FSC>100, FL2>100) were gated and the proportion of these cells staining intensely for PE (PE>1000) was calculated. Results are expressed as the percentage intensely stained cells after subtraction of background fluorescence for unstained cells incubated with rat IgG (% PE>1000). For IL-4, the geometric mean fluorescence (GMF) was measured for stained cells and the background subtracted.

Lung histology

Immediately after treatment, the lungs of 2-4 mice from each treatment group were fixed in 10% neutral buffered formalin and sent for independent analysis. Lungs were embedded in paraffin, sectioned and stained with haematoxylin and eosin (H&E). The slides were evaluated for peribronchial inflammation and scores were assigned on a scale of 0-4, corresponding to a score of normal to severe, respectively (9).

Whole body plethysmography

In this study, AHR was measured using unrestrained whole body plethysmography (8) with a four-chamber system (Buxco, Sharon, Connecticut, USA). Mice were first challenged with intranasal antigen and allowed to recover for 2 hours before being placed into the chambers and their breathing monitored for 10min. When acclimatized, their baseline response was measured for 5min. The mice were then subjected to 1min of aerosolised PBS, followed by progressively increasing doses of methacholine (5, 10, 20, 30, 40mg/ml PBS). Responses are recorded for 5min in every case with a short interval between to allow return to baseline. Responses were quantified using the measurement of enhanced expiratory pause (Penh).
Each group contained 4-8 mice. Penh is measured as the average percentage increase over the baseline value for mice in each group. Results are presented as the average percentage elevation in Penh after a challenge of methacholine. In order to determine if treatment produced a lasting effect, mice from the Der p experiment were re-challenged with allergen extract alone given intranasally, 4 days after completion of treatment.

Statistics

Results are the average for 4-8 mice/group and error bars are ± SEM. Significance was determined by Student's two tailed t-test. Significance was accepted for $p<0.05$.

Mouse model of pathogenic lung infection

Pretreatment with CMP and pathogen challenge

NMRI female mice were anaesthetized with ketamine/ xylazine and treated daily for three days with 250µg/µl CMP or 20µl PBS as control. On day 0, mice were anaesthetized with ketamine/xylazine and challenged with *P. aeruginosa* 2×10^6 intranasally. Animal survival was measured over 10 days.

Mouse model of bacterial infection

S. pneumoniae preparation

S. pneumoniae serotype 2, strain D39, was obtained from the National Collection of Type Cultures, London, UK (NCTC 7466). Bacteria were identified as pneumococci prior to infection by Gram stain, catalase test, haemolysis on blood agar plates and by optochin sensitivity. The capsular polysaccharide serotypes were confirmed by the Quellung reaction. For use in *in vivo* infection experiments, pneumococci were cultured and passaged through mice as described previously (10) and subsequently stored at -70°C. When required, suspensions were thawed at room temperature and bacteria harvested by centrifugation before re-suspension in sterile PBS.

Infection of mice

Female MF1 out-bred mice, nine weeks old and weighing 30-35 g (Harlan Olac, Bichester, UK), were lightly

anaesthetised with 2.5% (vol/vol) fluo-thane (AstraZeneca, Macclesfield, UK) over oxygen (1.5-2 litre/min). 50 µl PBS containing 1 x 10^6 cfu *S. pneumoniae* was administered into the nostrils of mice. The inoculum was confirmed by plating out on blood agar plates following infections. At hourly intervals following infection pre-selected groups of mice were deeply anaesthetised with 5% (vol/vol) fluo-thane before collection of blood by cardiac puncture. Following this procedure, the mice were killed immediately by cervical dislocation. The lung were removed into 10ml of sterile distilled water, weighed and then homogenised in a Stomacher-Lab blender (Seward Medical, London, UK). Viable counts in homogenates and in blood were determined as before (10). The presence of type 2 polysaccharide capsule was confirmed by the Quellung reaction. These mice did not have detectable levels of anti-type 2, anti-type 3 or anti-pneumolysin antibodies.

<u>Treatment Strategy</u>

On day -1, -2 and -3, mice were given 250µg CMP in 50µl sterile PBS, administered intranasally to anaesthetised mice. On day 3, mice were given the intranasal challenge with *S. pneumoniae* and sacrificed at hourly intervals.

<u>Cytokine production in human leukocytes</u>

<u>Effect of CMP administration on human monocytes</u>

Blood was obtained from three healthy donors and 10U/ml heparin added as an anticoagulant. Aliquots of 0.6ml were incubated with 10µg/ml brefeldin A (BFA), an inhibitor of protein secretion from cells and 5ng/ml LPS derived from *Salmonella Minnesota*, with or without 0.1µg/ml CMP. Incubation was at 37°C for 6h. After incubation, red

blood cells were lysed with ammonium chloride buffer and re-suspended in FACS staining buffer. Cell surface markers CD14 and CD16 were stained with fluorescent-labelled antibody, after cell permeabilization with saponin. Specificity of staining for TNFα was demonstrated by performing the intracellular cytokine staining in the presence of a 9-fold excess of recombinant human TNFα. After FACS analysis of the stained cells, the CD14 or CD14/CD16 populations were gated and results are given as the median fluorescence intensity (MFI) for TNFα staining after subtraction of the background staining for each donor.

Effect of CMP administration on human T-lymphocytes

Blood aliquots from two donors were incubated with BFA and 5ng/ml PMA + 0.25μM Ionomycin as a T-cell activator in the presence or absence of 0.1μg/ml CMP for 6h at 37°C. T-cells were stained for surface marker CD2 and stained for IFNγ by intracellular cytokine staining.

Results

Example 1

The effect of treatment with CMP on blood eosinophilia of animals challenged with Afu, is shown in Figure 1. Groups had received treatment for 4 days and measurements were made on day 5. The sample size was 4-5 mice/group. Error bars ± SEM. The results indicate that treatment with CMP resulted in a drop in the blood eosinophilia level to ca 0.3 x 10^6/ml, compared with test animals treated with PBS which exhibited blood eosinophilia levels of ca 0.7 x 10^6/ml.

Example 2

The effect of treatment with CMP on peripheral blood eosinophilia of mice challenged with Der p and Afu is shown in Figure 2. Groups were challenged daily with Afu or Der p extract, given intranasally, followed by intranasal treatment with 4 daily doses of 25μg CMP. PBS represent un-sensitised mice treated with PBS. Der-PC represents sensitised mice treated with a particulate control (PC) of latex beads. The sample size was 4-8 mice/group. Error bars ± SEM. Peripheral blood eosinophilia was reduced by 36% in the Der p model and 58% in the Afu model ($p<0.05$).

Example 3

A comparison of the effect of treatment with CMP on serum IgE levels of mice challenged with Afu, is shown in Figure 3. Groups were treated for 5 days and measurements were made on blood collected 3 days later. The sample size was 4-5 mice/group. Error bars ± SEM. The results indicate that serum IgE levels 3 days after intranasal treatment with CMP (Afu-Chitin) are less than 5μg/ml IgE, compared with 24μg/ml IgE in sensitised animals treated with PBS (Afu-PBS). PBS represent un-sensitised mice treated with PBS.

Example 4

The effect of treatment with CMP on serum IgE levels of mice challenged with Afu is shown in Figure 4. Groups were challenged daily with Afu extract, given intranasally, followed by intranasal treatment with 5 daily doses of 17μg CMP (A-CMP1) or PBS (A-PBS1) given 1 hour afterwards. Mice were re-challenged with 3 daily intranasal doses of allergen extract given alone the

following week and blood IgE levels re-measured (A-PBS2, A-CMP2). The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a significant reduction in serum IgE ($p<0.0005$) which was maintained following re-challenge with allergen, one week later ($p<0.0005$).

Example 5

The effect of treatment with CMP on serum IgE levels of mice challenged with Der p, is shown in Figure 5. Groups were challenged daily with Der p extract, given intranasally, followed by intranasal treatment with 5 daily doses of PBS (DerPBS), 25μg CMP (DerCMP) or 25μg or a particulate control of latex beads (DerPC). PBS represents un-sensitised mice treated with PBS, given 1 hour later. The sample size was 4-8 mice/group. Error bars ± SEM. Treatment with 5 daily doses of 25μg CMP produced a significant decrease in total serum IgE ($p<0.005$), measured 4 days after treatment.

Example 6

A comparison of the effect of treatment with CMP or PBS on serum IgG1 levels of mice challenged with Afu, is shown in Figure 6. Groups were challenged and treated daily for 5 days with either PBS or 25μg CMP given intranasally and measurements were made on blood collected 3 days later. The sample size was 4-5 mice/group. Error bars ± SEM. Sensitised animals challenged intranasally with Afu allergen extract, followed by intranasal treatment with CMP (Afu-CMP) showed a four fold decrease in serum IgG1 levels relative to sensitised animals treated with PBS (Afu-PBS). PBS represent un-sensitised mice treated with PBS.

Example 7

The effect of treatment with CMP on Afu specific IgG1 levels of mice challenged with Afu, is shown in Figure 7. Groups were challenged daily with Afu extract, given intranasally, followed by intranasal treatment with 5 daily doses of 17μg CMP (A-CMP1) or PBS (A-PBS1) 1 hour afterwards. Mice were re-challenged with 3 doses of allergen extract alone the following week and blood IgG1 levels re-measured (A-PBS2, A-CMP2). The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a significant reduction in Afu-specific IgG1 ($p<0.001$) which was maintained on re-challenge with allergen, one week later ($p<0.01$).

Example 8

The effect of allergen re-challenge on AHR of mice challenged with Afu, is shown in Figure 8. Groups were treated with intranasal challenges of Afu allergen followed by intranasal treatment with PBS (Afu-PBS) or 20ug CMP (Afu-CMP) repeated daily for 4 days. Mice were re-challenged with allergen alone and AHR measured in response to provocation with 30mg/ml of nebulized methacholine. PBS represent un-sensitised mice treated with PBS. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate that AHR was significantly reduced ($p<0.01$) in animals treated with CMP. These mice showed only a 110% increase in Penh over control mice when challenged with methacholine, compared to a 240% increase for PBS treated mice.

Example 9

Airway hyperresponsiveness of mice challenged with Afu, in response to a 20mg/ml challenge of nebulized methacholine, is shown in Figure 9. Groups were given 4

daily doses of PBS or 25µg CMP intranasally. The sample size was 4-8 mice/group. Error bars ± SEM. A significant reduction in AHR was observed in the CMP treated group ($p<0.01$). Treatment with the particulate control of latex beads (A-PC) did not reduce AHR.

Example 10

A dose response of treatment groups challenged with Der p to nebulized methacholine is shown in Figure 10. Groups were first given an intranasal challenge of Der p extract followed by intranasal treatment with PBS (Der-PBS) or 25µg CMP (Der-CMP), repeated for 4 days. Mice were re-challenged with Der p 4 days after completion of the challenge/treatment course. AHR was measured in response to different doses of nebulized methacholine. PBS represent un-sensitised mice treated with PBS. The sample size was 4-8 mice/group. Error bars ± SEM. The results indicate a reduced AHR to all concentrations of methacholine tested.

Example 11

AHR of mice challenged with Der p in response to nebulized methacholine is shown in Figure 11. Groups were treated for 3 days with 25µg CMP intranasally, preceded by allergen challenge (Der p(0)) and re-challenged with allergen alone 4 days after completion of treatment with a total of 4 daily doses of 25µg CMP preceded by allergen challenge (Der-CMP(4)). PBS represent un-sensitised mice treated with PBS. Results are expressed as the elevation of Penh and show a significant reduction in AHR on the fourth day of treatment (Der-CMP(0), $p<0.001$) and after re-challenge 4 days after treatment (Der-CMP(4), $p<0.001$).

Example 12

AHR of Der p (H=Der p) sensitised mice treated with CMP is shown in Figure 12. Groups were treated for 4 days with four different doses of CMP (5-40μg). On day 4 mice were challenged with Der p and treated 1-2h later with CMP or a control treatment of the CMP supernatant, free of any CMP, from a 25μg/ml suspension (Psn). AHR was measured after exposure to 100mg/ml nebulized methacholine for 1.5min. P represents non-sensitised mice. Results show all doses of CMP were equally effective and suggest that a dose of five fold lower than that used in previous experiments can be used to prevent an allergic response in this model.

Example 13

Lung sections stained with haematoxylin and eosin, illustrating the differences in the degree of inflammation and obstruction of airways after treatment of Afu sensitised mice with CMP, are shown in Figure 13. The peribronchial inflammation of allergen challenged mice treated with PBS gave an average score of 2.5 compared with a score of 1 for CMP treated mice also challenged with allergen. This represents a 60% reduction in allergen induced inflammation. Non-sensitised mice treated with PBS gave a score of 0. Figure 13a shows normal mouse lung after treatment with PBS, 13b shows allergic lung treated with PBS and 13c shows allergic lung after intranasal treatment with 4 daily doses of 25μg CMP.

Example 14

The effect of treatment on IL-12, IFN-γ, TNF-α and IL-4

levels in Der-p and Afu challenged mice is shown in Table 1a. Groups were given 4 daily doses of allergen extract followed by intranasal treatment with 25μg CMP or a non-specific particulate control of polystyrene microbeads (PC). Cytokine producing activity was assessed by measuring the proportion of highly stained cells positive for the respective anti-cytokine antibody labelled with phycoerythrin. Results are shown ± SEM. IL-12 was significantly elevated by 77% (Der-CMP, $p<0.005$) in the Der p model and elevated by 43% (Afu-CMP) in the Afu model. The particulate control did not elevate IL-12 levels. IFN-γ was significantly elevated by 41% (Der-CMP, $p<0.05$) in the Der p model and by 22% (Afu-CMP, $p<0.005$) in the Afu model. TNF-α was significantly elevated by 44% (Der-CMP, $p<0.05$) in the Der p model and by 22% (Afu-CMP, $p<0.05$) in the Afu model.

The effect of treatment on GM-CSF levels in Der p challenged mice is shown in Table 1b. Mice were treated with 40μg CMP. GM-CSF was measured by intracellular cytokine staining and flow cytometry. Treatment with CMP produced a significant ($p<0.05$) decrease in GM-CSF relative to treatment with PBS alone and approached the levels seen in non-allergic naïve mice.

Comparison of the geometric mean fluorescence (GMF) of spleen cells stained for IL-4 (Table 1c) showed a decrease of 34% (Der-CMP) in the Der p model and 27% (Afu-CMP) in the Afu model. No decrease was observed with the particulate control.

<u>Example 15</u>

The effect of pre-treatment with CMP on *P. aeruginosa* infected mice is shown in Figure 14. Mice were pre-treated for three days with 250μg CMP in PBS,

intranasally, before intranasal infection with 2x10° *P. aeruginosa*. Animal survival was measured for 10 days. The experiment was performed twice and the results combined. One day after pathogen challenge 40% of the control treated mice had died whereas, in contrast, the CMP treated mice showed a 100% survival rate. Three days after pathogen challenge, 70% of the control treated mice had died, whereas the CMP treated mice exhibited a 80% survival rate. No change in these survival rates was observed up to 10 days after infection.

Example 16

The effect of 250μm CMP pre-treatment on lung clearance of *S. pneumoniae* in mice is shown in Figure 15. No difference was observed between PBS and CMP treated mice from 0-12h; however at the 24h time point CMP treated mice had significantly lower bacterial cfu in the lungs ($p<0.001$).

Example 17

The effect of 250μm CMP pre-treatment on the appearance of *S. pneumoniae* in the blood of infected mice is shown in Figure 16. No bacteria were observed at the 12h time point and blood bacteraemia was significantly less at 24h ($p<0.005$) and 48h, suggesting that CMP is protective in the lungs by 12-24h.

Example 18

The effect of 0.1μg/ml CMP on TNFα production in LPS activated CD14 human monocytes is shown in Figure 17. There was very little production of TNFα by monocytes incubated with 0.1μg/ml CMP alone. However, incubation of blood with 0.1μg/ml CMP supplemented with 5ng/ml LPS as a

monocyte activator resulted in a significant ($p<0.001$) elevation in TNFα production measured in the $CD14^+$ monocytes when compared to the cells stimulated with LPS alone.

Example 19

The effect of 0.1μg/ml CMP on TNFα production in LPS activated CD14/CD16 proinflammatory human monocytes is shown in Figure 18. There was little production of TNFα in proinflammatory monocytes incubated without CMP or LPS. Incubation with 0.1μg/ml CMP supplemented with 5ng/ml LPS resulted in an elevation in TNFα production of approximately 350MFI, when compared to the cells stimulated with LPS alone.

Example 20

The production of IFNγ from PMA/Ionomycin stimulated human T-lymphocytes by 0.1μg/ml CMP is shown in Figure 19. The addition of CMP elevated the production of IFNγ by 20% from PMA/Ionomycin stimulated $CD2^+$ T-lymphocytes cultured *in vitro*. The results indicate that CMP are able to promote the activity or proliferation of Th1 lymphocytes.

Table 1

a)

Cytokine	Percentage staining above isotype control (%PE>1000)							
	PBS	Der-PBS	Der-CMP	Der-PC	PBS	Afu-PBS	Afu-CMP	Afu-PC
IL-12	3.3±0.1	0.9±0.1	4.2±0.1	1.0±0.1	2.0±1.0	11.5±6.0	19.0±7.0	12.0±4.0
IFN-γ	11.6±2.7	5.8±1.2	9.8±1.6	-	30.0±3.0	24.0±2.0	32.0±2.0	27.0±1.0
TNF-α	4.0±2.5	4.2±1.0	10.0±2.5	-	6.5±2.0	12.5±2.5	16.5±3.0	-

b)

Cytokine	Percentage staining above isotype control (%PE>1000)			
	Naive	Der-PBS	Der-CMP	Der-PC
GM-CSF	0.47±0.05	0.75±0.08	0.48±0.07	-

c)

Cytokine	Geometric mean fluorescence							
	PBS	Der-PBS	Der-CMP	Der-PC	PBS	Afu-PBS	Afu-CMP	Afu-PC
IL-4	16.5±3.5	29.0±9.0	19.0±3.0	26.5±7.5	16.5±3.5	13.0±1.0	7.0±2.0	-

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The references mentioned herein are all expressly incorporated by reference.

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Claims:

1. Use of a chitin microparticle preparation (CMP) for the preparation of a medicament for the treatment of allergy, wherein the medicament is delivered intranasally or by inhalation and the chitin microparticles have an average diameter of less than 10μm.

2. The use of claim 1, wherein the allergy is seasonal respiratory allergies; allergy to aeroallergens; allergy treatable by reducing serum IgE and eosinophilia; asthma; eczema; food allergy; dermatitis; or the treatment of allergy by allergic desensitisation.

3. The use of claim 2, wherein the aeroallergen is house mite dust, fungal spores, grass pollens, tree pollens or animal danders.

4. The use of claim 2 wherein the dermatitis is atopic dermatitis.

5. The use of claim 1, wherein the chitin microparticle composition is for allergic desensitisation and further comprises an allergen.

6. The use of claim 5, wherein the allergen is a food allergen.

7. The use of claim 6, wherein the food allergen is found in milk, wheat, gluten, eggs, nuts or shellfish.

8. The use of claim 1, wherein the medicament is used to treat an animal.

9. The use of claim 8, wherein the animal is a horse and the allergy is asthma or is associated with recurrent lung infection.

10. Use of a chitin microparticle (CMP) preparation for the preparation of a medicament for the treatment of conditions benefiting from up-regulation of the cell-mediated immune system, wherein the medicament is administered intranasally or by inhalation and the chitin microparticles have an average diameter of less than 10μm.

11. The use of claim 10, wherein the condition benefiting from up-regulation of the cell-mediated immune system is a bacterial infection, a fungal infection or a viral infection.

12. The use of claim 11, wherein the infection is an ear, nose, throat or lung infection.

13. The use of any one of claims 10 to 12, wherein the medicament is used for the treatment of a patient at risk of developing an infection.

14. The use of claim 13, wherein the patient at risk is an elderly person, a premature baby, an infant, a transplantation patient, an immunosuppressed patient, a chemotherapy patient, a hospital patient at risk of opportunistic infection, a patient on a ventilator, a cystic fibrosis patient or a patient with AIDS.

15. The use of any one of claims 11 to 14, wherein the condition is a bacterial infection by *Pseudomonas aeruginosa,* a *Streptococcus* species, *Haemophilus*

influenza, Klebsiella pneumoniae, Yersinia enteocolitica, Salmonella, Listeria, a *Mycobacteria* species or a parasitic infection.

16. The use of claim 15, wherein the *Streptococcus* species is *Streptococcus pneumoniae, Streptococcus pyrogenes* or *Streptococcus agalactiae.*

17. The use of claim 15, wherein the *Mycobacterial* species is *Mycobacterium tuberculosis* or *Mycobacterium leprae.*

18. The use of claim 15, wherein the parasitic infection is an infection by a *Leishmania* species and *Schistosoma* species.

19. The use of any one of claims 11 to 14, wherein the condition is bacterial pneumonia such as ventilator-associated pneumonia or cystic fibrosis associated infections.

20. The use of any one of claims 11 to 14, wherein the condition is Otitis media.

21. The use of any one of claims 11 to 14, wherein the fungal infection is invasive pulmonary aspergillosis and invasive pulmonary candidiasis, *Pneumocystis carinii* pneumonia, or a *Coccidioides* or *Crytococcus.*

23. The use of any one of claims 11 to 14, wherein the condition is a pulmonary viral infection.

24. The use of any one of claims 11 to 14, wherein the viral infection is caused by infection by respiratory

syncytial virus bronchiolitis, influenza virus, rhino virus or human immunodeficiency virus (HIV).

25. Use of a chitin microparticle preparation for the preparation of a medicament for the treatment of a condition by up-regulating of the activity of NK cells and/or secretion of IFN-γ by cells of the immune system, wherein the medicament is administered intranasally or by inhalation and the chitin microparticles have an average diameter of less than 10μm.

26. The use of claim 25, wherein the condition is cancer.

27. The use of claim 25, wherein the condition is lung cancer, lung carcinoma or nasal-pharyngeal carcinoma.

28. The use of claim 25, wherein the condition is a chronic lung disorder.

29. The use of any one of the preceding claims, wherein the medicament is administered prophylactically.

30. The use of any one of the preceding claims, wherein the chitin microparticles have an average diameter of less 5μm.

31. The use of any one of the preceding claims, wherein the chitin microparticles have an average size of at least 1μm in diameter.

32. The use of any one of the preceding claims, wherein the chitin microparticles are derived from the

exoskeletons of crab, shrimp, lobster, cuttlefish, and insects and fungi.

33. The use of any one of the preceding claims, wherein the chitin microparticles are obtainable by sonicating or milling purified chitin.

34. The use of any one of the preceding claims, wherein the chitin microparticles are obtainable by coating carrier particles with *N*-Acetyl-D-Glucosamine, chitin or a fragment thereof.

35. The use of any one of the preceding claims, wherein the medicament is administered to a patient in a therapeutically effective amount of between 0.01 and 100mg of active compound per kg of body weight.

36. The use of any one of the preceding claims wherein the medicament is administered to humans.

37. The use of any one of the preceding claims wherein the chitin microparticle preparation comprises one or more of a pharmaceutically acceptable excipient, a carrier, a propellant, a buffer, a stabiliser, an isotonicizing agent, a preservative or an antioxidant.

38. A delivery device for the administration of the chitin microparticles of any one of the preceding claims, comprising:

a) a reservoir of chitin microparticles having an average diameter of less than 10μm;

b) a delivery orifice adapted to locate in a patient's mouth or nose; and

c) a valve between the reservoir and the delivery

orifice such that the valve can be operated to control delivery of the chitin microparticles.

39. A composition comprising a chitin microparticle composition and an allergen, wherein the chitin microparticles have a diameter of less than 10μm.

40. The composition of claim 39, wherein the allergen is a food allergen.

41. The composition of claim 40, wherein the food allergen is an allergen found in milk, wheat, gluten or eggs.

42. A composition comprising a chitin microparticle composition and an allergen, wherein the chitin microparticles have a diameter of less than 10μm for use in a method of medical treatment.

43. A kit comprising:

(a) a chitin microparticle composition wherein the chitin microparticles have a diameter of less than 10μm; and

(b) an allergen;

for simultaneous or sequential administration to a patient.

Figure 1

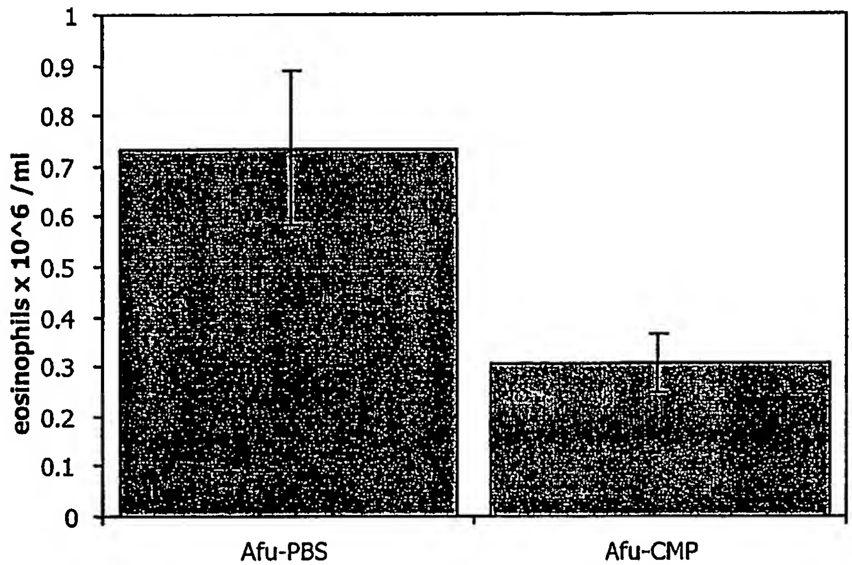


Figure 2

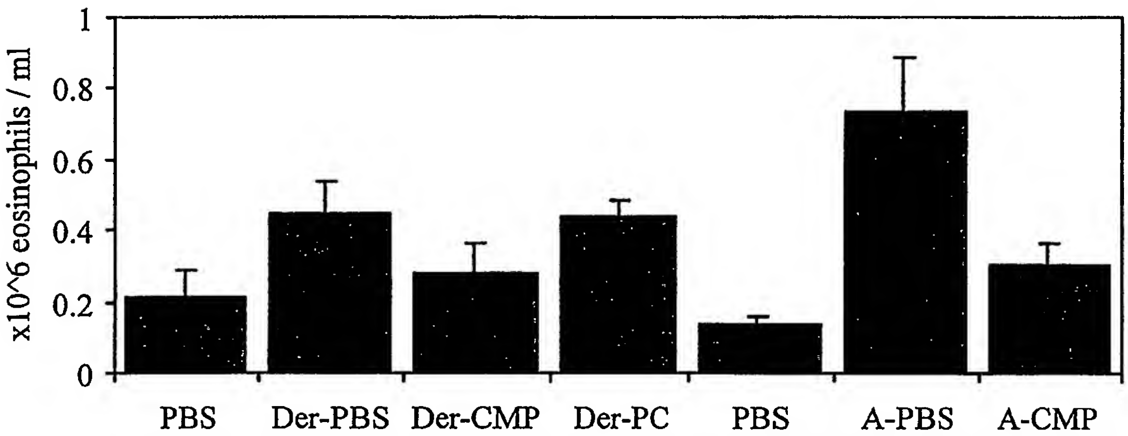

x10^6 eosinophils / ml
1
0.8
0.6
0.4
0.2
0
PBS
Der-PBS
Der-CMP
Der-PC
PBS
A-PBS
A-CMP

Figure 3

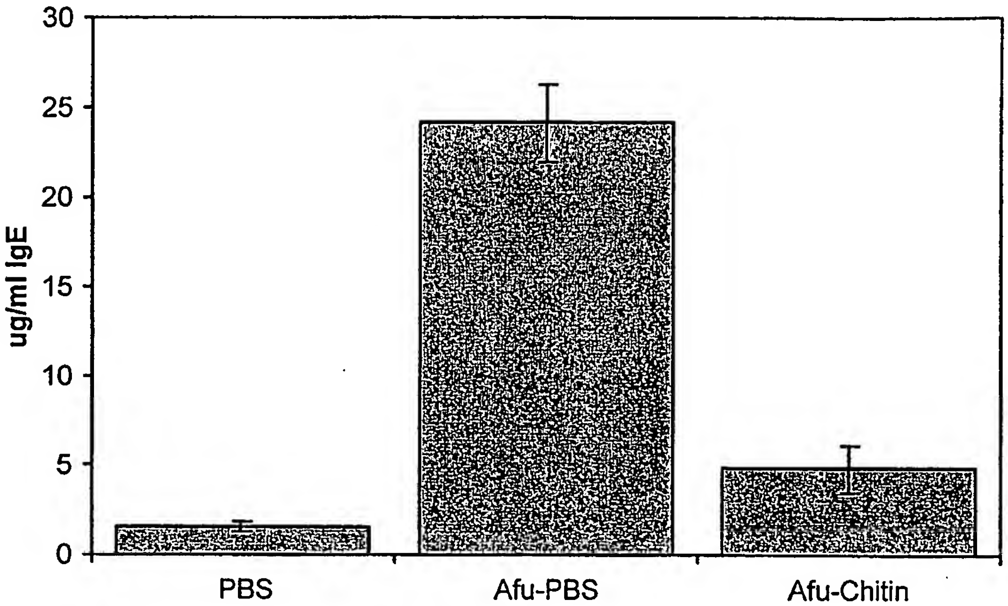


Figure 4

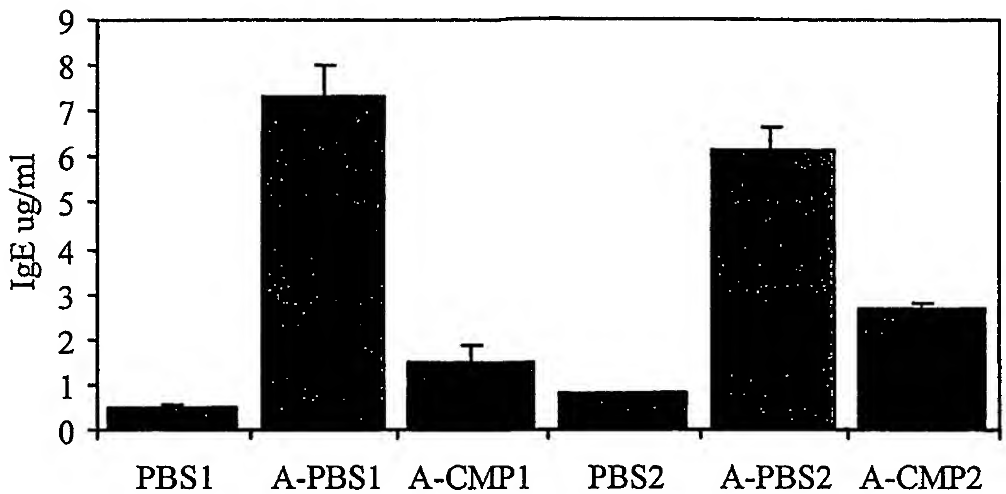


Figure 5

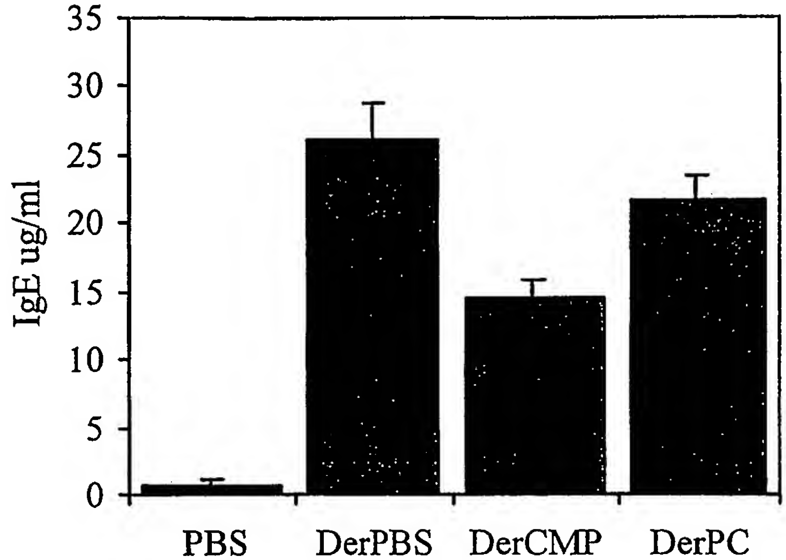


Figure 6

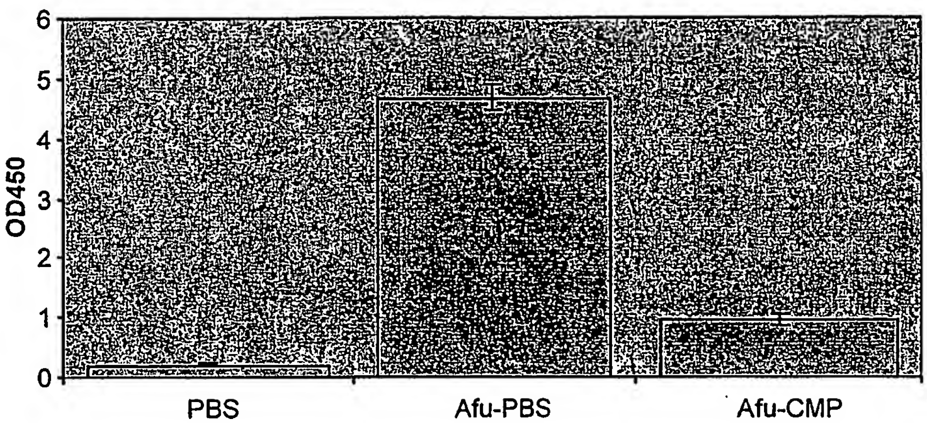


Figure 7

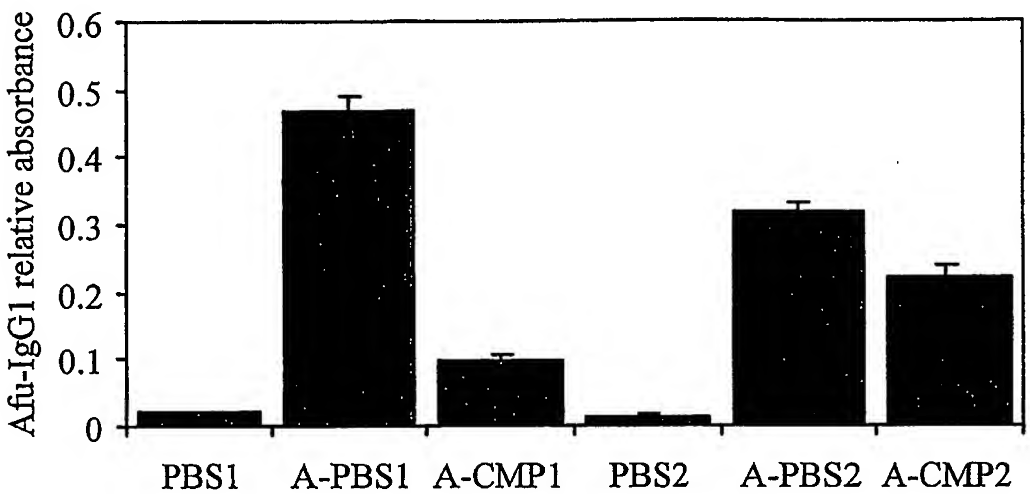

Afu-IgG1 relative absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
PBS1
A-PBS1
A-CMP1
PBS2
A-PBS2
A-CMP2

Figure 8

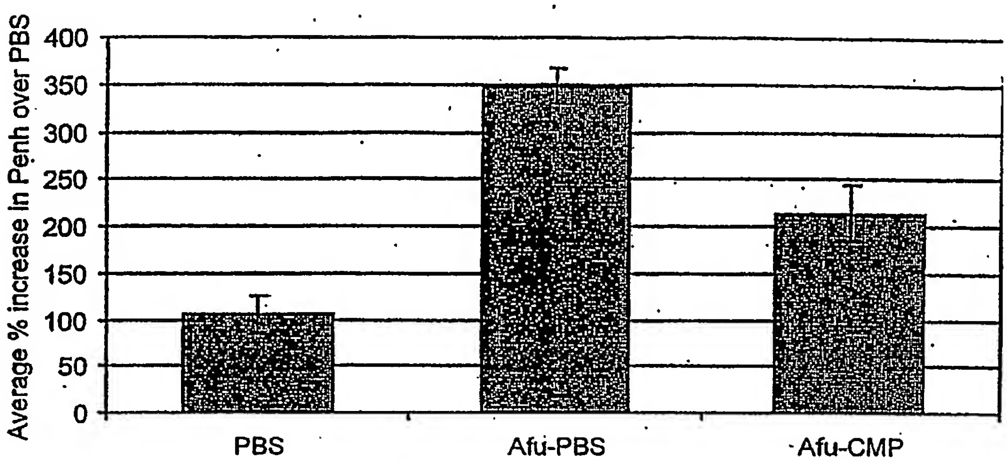

Average % increase in Penh over PBS
400
350
300
250
200
150
100
50
0
PBS
Afu-PBS
Afu-CMP

Figure 9

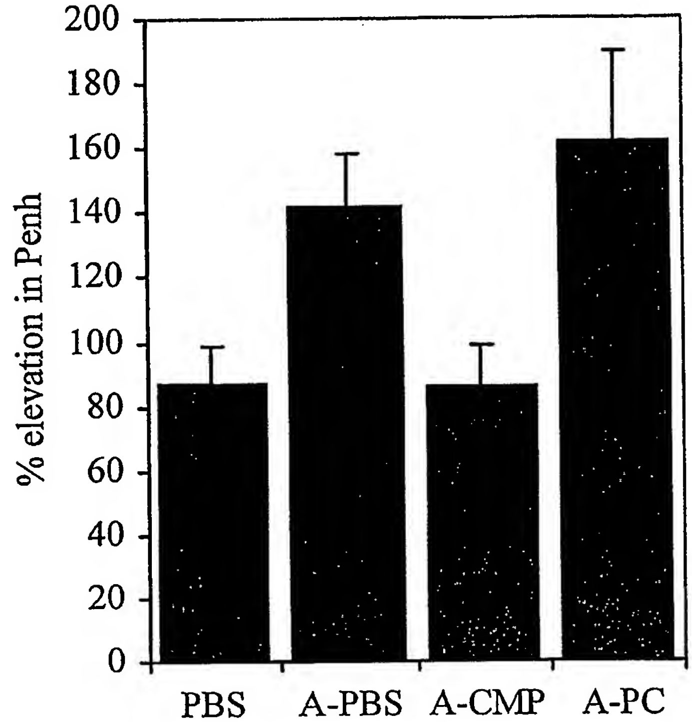

200
180
160
140
120
100
80
60
40
20
0
% elevation in Penh
PBS
A-PBS
A-CMP
A-PC

Figure 10

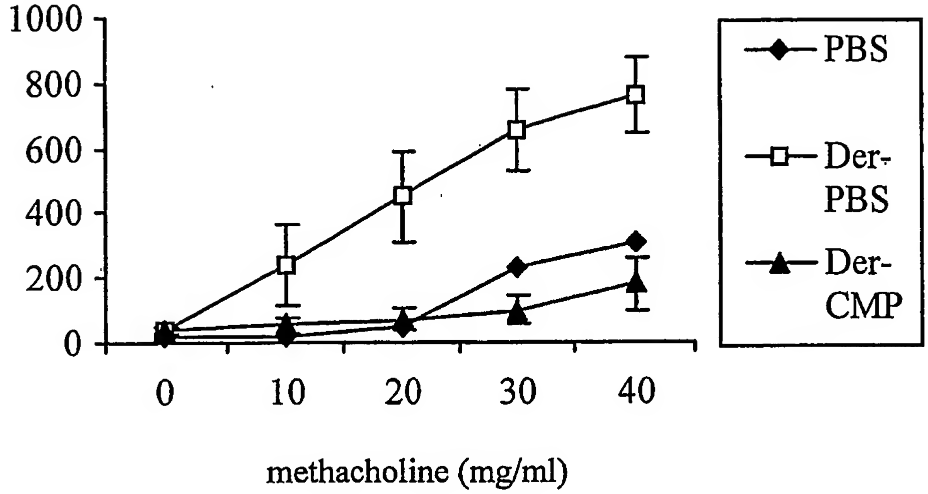

1000
800
600
400
200
0
0
10
20
30
40
methacholine (mg/ml)
PBS
Der-PBS
Der-CMP

Figure 11

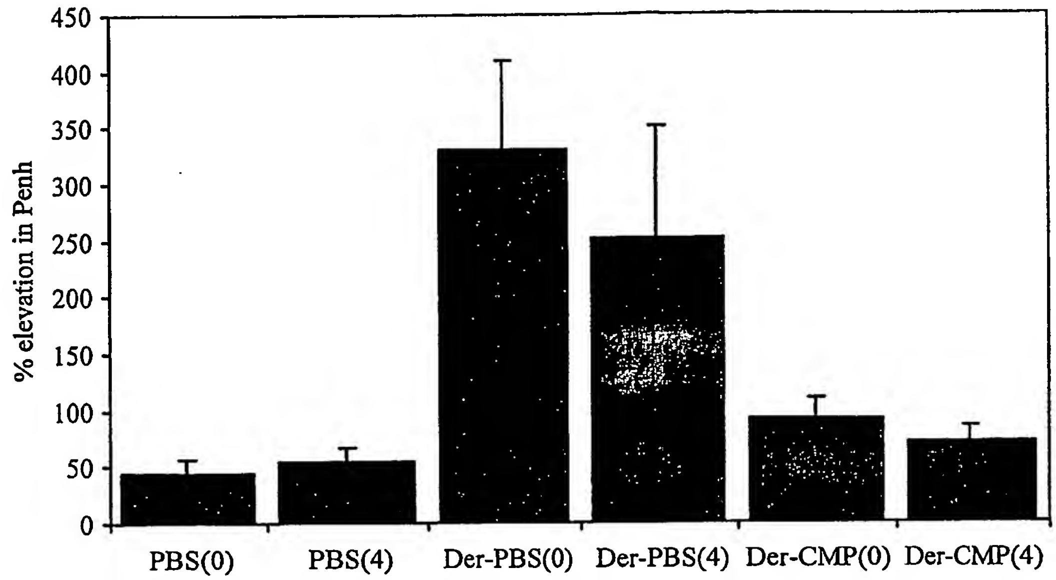

450
400
350
300
250
200
150
100
50
0
% elevation in Penh
PBS(0)
PBS(4)
Der-PBS(0)
Der-PBS(4)
Der-CMP(0)
Der-CMP(4)

Figure 12

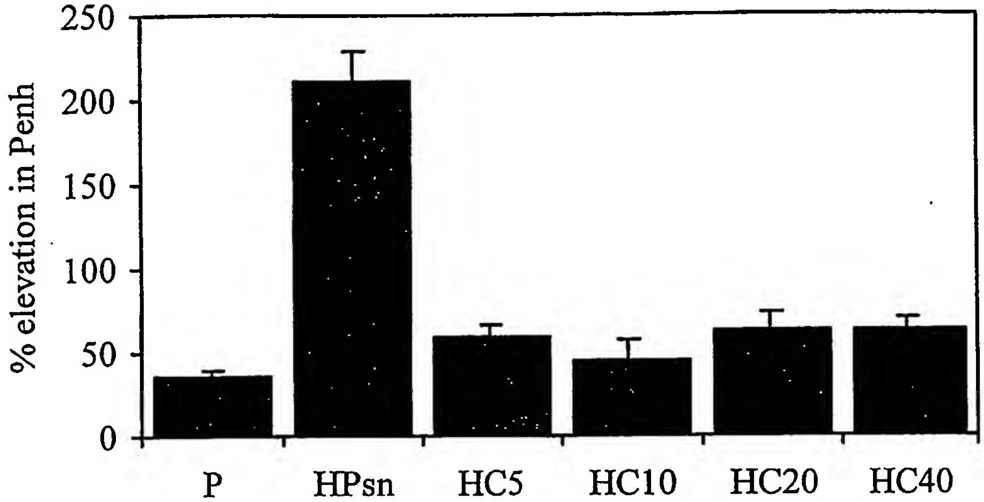

250
200
150
100
50
0
% elevation in Penh
P
HPsn
HC5
HC10
HC20
HC40

Figure 13

a

b

c

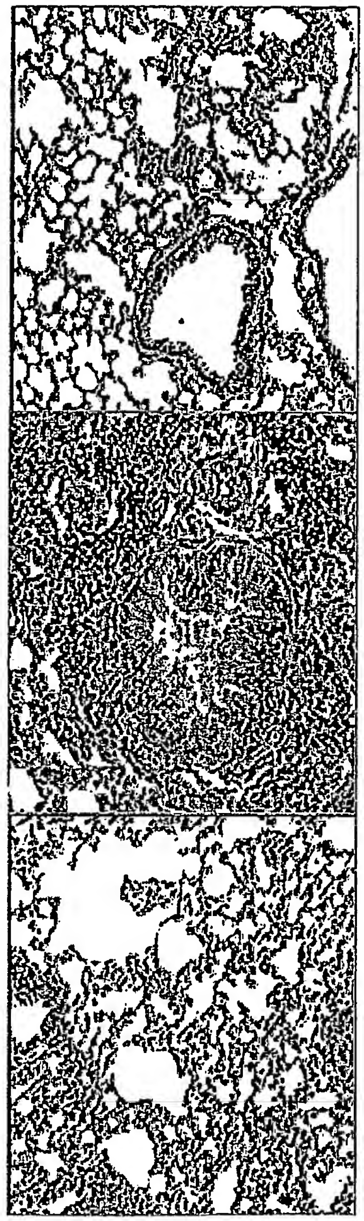


Figure 14

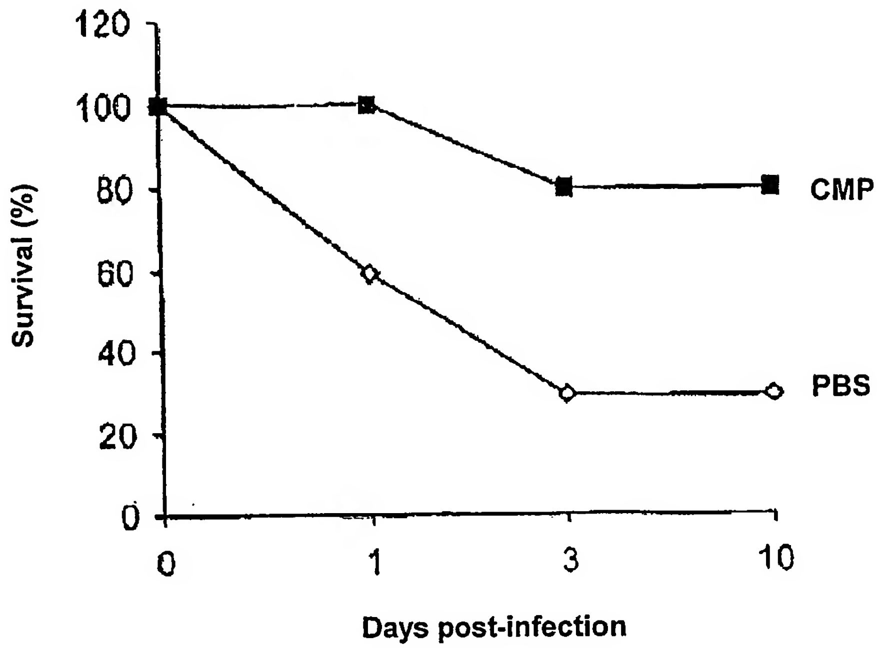

120
100
80
60
40
20
0
Survival (%)
CMP
PBS
0
1
3
10
Days post-infection

Figure 15

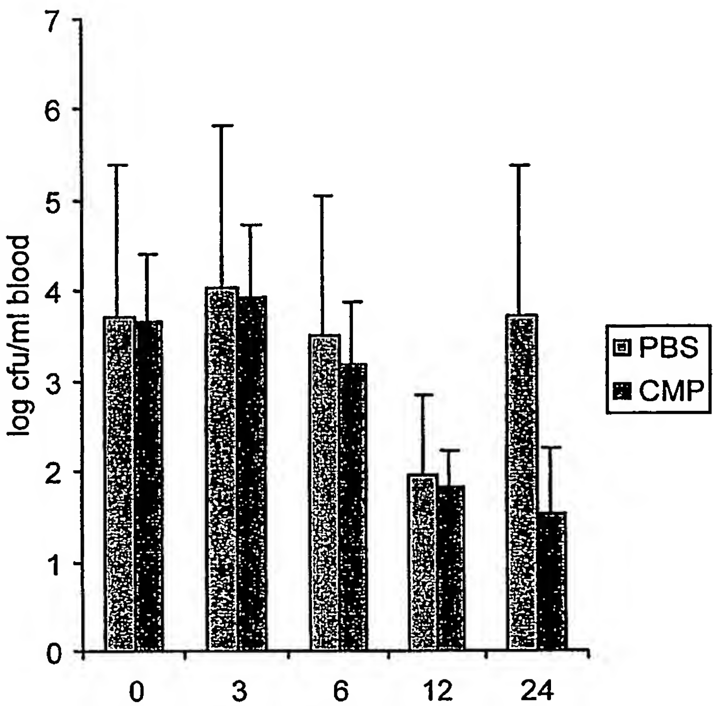


Figure 16

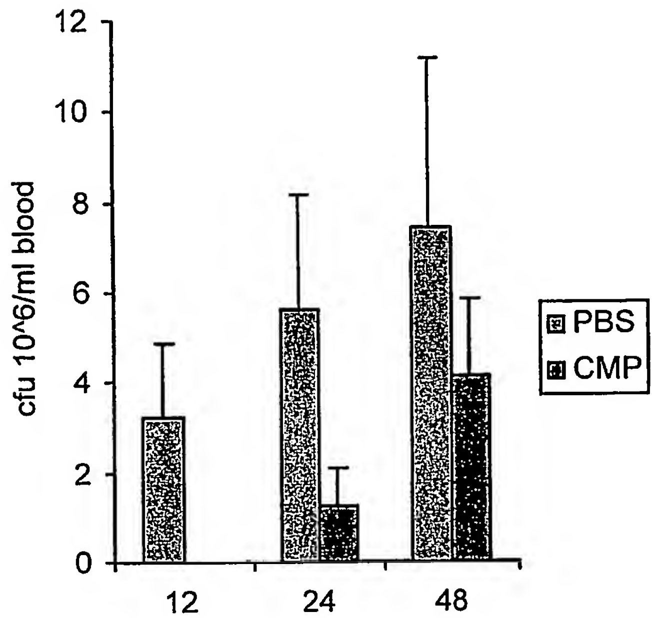


Figure 17

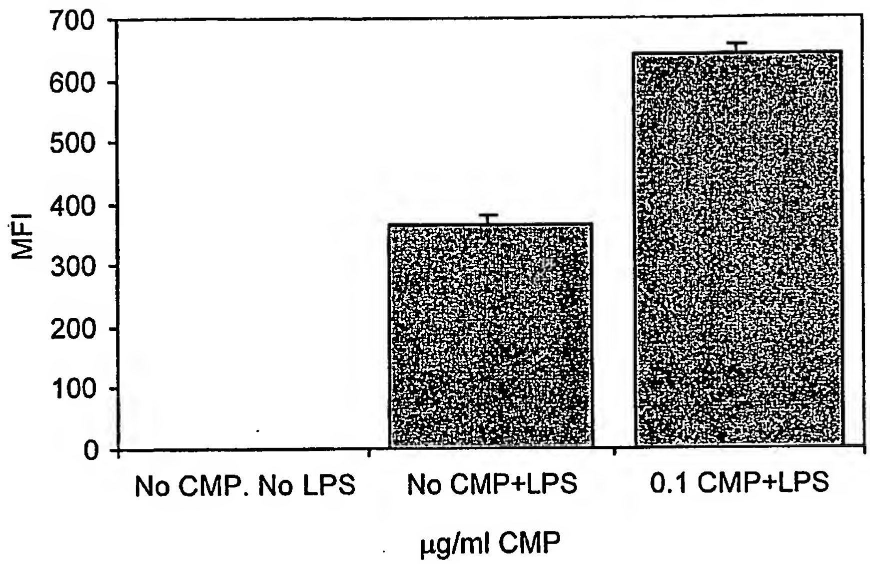

700
600
500
400
300
200
100
0
MFI
No CMP. No LPS
No CMP+LPS
0.1 CMP+LPS
µg/ml CMP

Figure 18

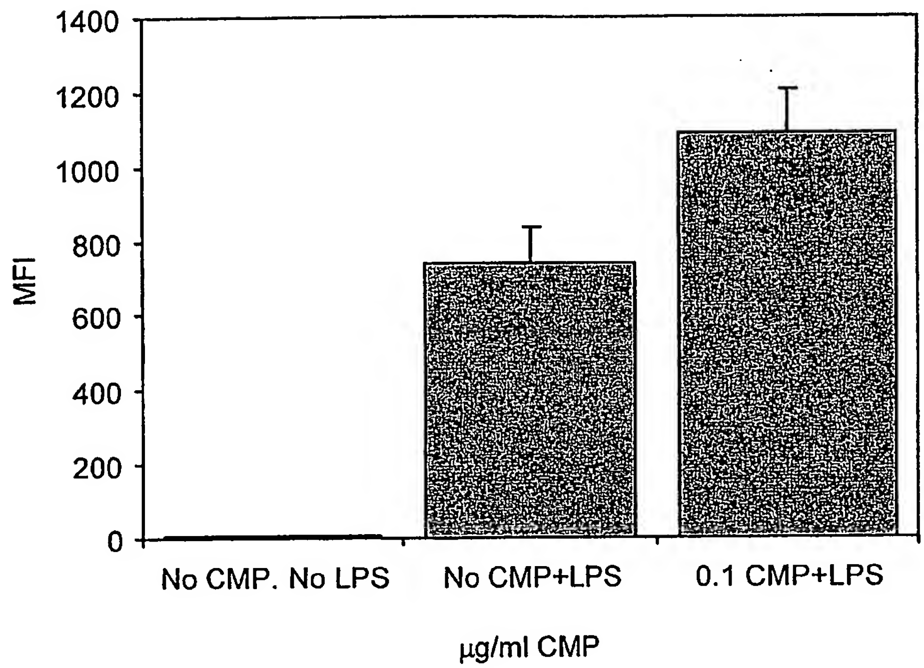
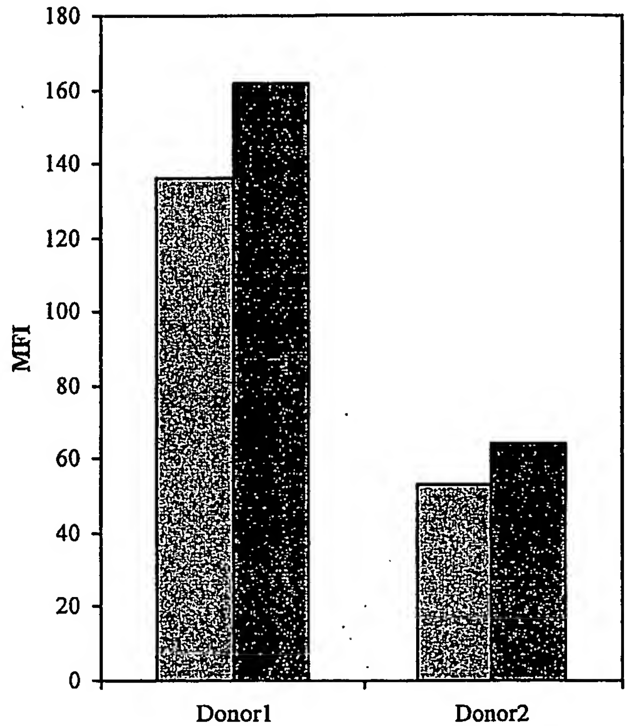

1400
1200
1000
800
600
400
200
0
MFI
No CMP. No LPS
No CMP+LPS
0.1 CMP+LPS
µg/ml CMP

Figure 19



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/03814

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K47/36 A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, CHEM ABS Data, BIOSIS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 591 441 A (GRISTINA ANTHONY G ET AL) 7 January 1997 (1997-01-07) cited in the application column 3, line 25 -column 4, line 2 column 10, line 2 - line 67 claims 1,4 ---	1-37
Y	US 5 585 106 A (GRISTINA ANTHONY G ET AL) 17 December 1996 (1996-12-17) column 2, line 25 - line 61 column 4, line 44 -column 5, line 4 column 12, line 26 - line 65 claims 1,3,9 ---	1-37

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2002	26 NOVEMBER 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rankin, R

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 292 513 A (GRISTINA ANTHONY G ET AL) 8 March 1994 (1994-03-08) column 3, line 25 - line 45 column 10, line 33 - line 56 column 11, line 11 - line 38 claims 1,3,5,7 ---	1-37
Y	SHIBATA Y ET AL: "Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD.: 1950) UNITED STATES 1 FEB 2000, vol. 164, no. 3, 1 February 2000 (2000-02-01), pages 1314-1321, XP002218084 ISSN: 0022-1767 cited in the application page 1319, column 2 ---	1-37
Y	SHIBATA Y ET AL: "Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice." INFECTION AND IMMUNITY. UNITED STATES MAY 1997, vol. 65, no. 5, May 1997 (1997-05), pages 1734-1741, XP002218085 ISSN: 0019-9567 cited in the application page 1734 -page 1741 ---	1-37
Y	NISHIMURA K ET AL: "EFFECT OF MULTIPOROUS MICROSPHERES DERIVED FROM CHITIN AND PARTIALLY DEACETYLATED CHITIN ON THE ACTIVATION OF MOUSE PERITONEAL MACROPHAGES" VACCINE, vol. 5, no. 2, 1987, pages 136-140, XP001109049 ISSN: 0264-410X page 136 -page 140 ---	1-37
Y	WO 97 20576 A (DANBIOSYST UK ;ILLUM LISBETH (GB)) 12 June 1997 (1997-06-12) page 1, line 1-10 page 6, line 8 - line 24 page 9 -page 26; examples claims 1,6,15,21-24 ---	1-37
Y	PATENT ABSTRACTS OF JAPAN vol. 018, no. 680 (C-1291), 21 December 1994 (1994-12-21) & JP 06 271470 A (ASAHI CHEM IND CO LTD), 27 September 1994 (1994-09-27) abstract --- -/--	1-37

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 01, 29 January 1999 (1999-01-29) & JP 10 279606 A (UNITIKA LTD), 20 October 1998 (1998-10-20) cited in the application abstract -----	1-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/03814

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-37
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 1-37

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy Rule 39.1(iv)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 02/03814

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5591441	A	07-01-1997	US	5292513	A	08-03-1994
			US	5585106	A	17-12-1996
			US	5770234	A	23-06-1998
			CA	2135784	A1	25-11-1993
			EP	0671952	A1	20-09-1995
			JP	8501062	T	06-02-1996
			WO	9323079	A1	25-11-1993
US 5585106	A	17-12-1996	US	5591441	A	07-01-1997
			US	5292513	A	08-03-1994
			US	5770234	A	23-06-1998
			CA	2135784	A1	25-11-1993
			EP	0671952	A1	20-09-1995
			JP	8501062	T	06-02-1996
			WO	9323079	A1	25-11-1993
US 5292513	A	08-03-1994	CA	2135784	A1	25-11-1993
			EP	0671952	A1	20-09-1995
			JP	8501062	T	06-02-1996
			WO	9323079	A1	25-11-1993
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			AU	705452	B2	20-05-1999
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